Study of introgression lines of common wheat *Triticum aestivum/Triticum miguschovae* for resistance to leaf rust

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Abstract: The evaluation of resistance to leaf rust, the cytological and molecular analysis of 106 introgression lines of common wheat obtained on the basis of the synthetic form *Triticum miguschovae* Zhir were performed. Cytological stability of lines was established. 53 lines with high and 23 with medium resistance to leaf rust were selected. Two lines with the Lr39 gene and 26 lines with the Lr50 gene were identified with the use of molecular markers. Other resistant lines can presumably carry genes for resistance to leaf rust other than Lr39 or Lr50.

Key words: *T. aestivum*; *T. miguschovae*; introgression lines; resistance to leaf rust; cytological analysis; molecular markers.

1. Introduction

Leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Erikss.et Henn) is one of the most widespread and harmful diseases of wheat. The most effective way to control the infestation is by breeding resistant varieties. This work assumes the presence of a sufficient diversification of effective genes for resistance, including new ones. Of great interest are the species *Triticum timopheevii* Zhuk. (GGAA) and its natural mutant *T. militinae* Zhuk. et Migush., and *Aegilops tauschii* Coss. (DD) as sources of resistance to diseases (Friebe et al., 1996; McIntosh et al., 2005). The synthetic form *T. miguschovae* (GGA^tA^tDD) was used as a "genetic bridge' to transfer leaf rust resistance from these species to common wheat, where the D genome from *Ae. tauschii* was added to the AG genomes from *T. militinae* (Zhirov, 1980). To date, a large number of introgression lines and six varieties of winter common wheat have been obtained with the use of this form (Davoyan et al., 2012).

This article presents the results of the evaluation of 106 *T. aestivum/T. miguschovae* introgression lines of common wheat for resistance to leaf rust, cytological stability and the identification of previously transferred effective genes, *Lr39* and *Lr50*.

2. Materials and methods

106 BC_2F_{22}-BC_4F_{16} introgression lines of common wheat, obtained from crossing *T. miguschovae* with the varieties 'Bezostaya1', 'Kavkaz' and 'Skiphyanka' (MB, MK and MKC lines) susceptible to leaf rust were used as objects of research.

The cytological analysis was performed by studying chromosome paring in metaphase I of meiosis.

The reaction of plants to leaf rust infestation was evaluated using the international scale by Maines and Jackson in the field (Mains, Jackson, 1926). The plants with reaction types from 0 to 2 were classified as resistant. Plants with intermediate reaction types, between 0 and 1, were designated as 01. Plants with reaction types 3 and 4 were considered susceptible.

DNA of wheat was extracted from 5-7-day-old bleached-out sprouts using a method by Plaschke with the coauthors (1995). Identification of genes was done with the use of molecular marker *GDM-35* for *Lr39* gene (Huang et al., 2001) and the *GDM87* and *WMS382* markers for the *Lr50* gene (Brown-Guedira et al., 2003). Wheat lines KS86WGRC02 and KS96WGRC36 with genes for resistance to leaf rust, *Lr39* and *Lr50*, respectively, were used as positive controls for identification of known genes.

3. Results and discussion

As the main aim of the work was to transfer resistance to diseases from *T. miguschovae* to common wheat, the lines were evaluated for resistance to one of the most widespread and harmful of them, leaf rust. The characteristics of the population of lines assessed for resistance to this pathogen in 2016–2018 is presented in Table 1. The assessment of the lines revealed their difference in resistance to leaf rust in all combinations.

Highly resistant lines with reaction types 01 and 1, medium resistant (reaction type 2), medium susceptible (reaction type 3) and highly susceptible (reaction type 4) lines were identified.

Fifty-three lines showed high resistance to leaf rust. 23 lines were medium resistant and 22 lines were susceptible to a local population of leaf rust. Also, 8 lines with a heterogeneous reaction type were revealed, where single pustules with treaction type 3 appeared on the background of high resistance (reaction type 1). Wide polymorphism of lines in resistance to leaf rust can indicate different introgressions of genetic material from *T. miguschovae* to the common wheat genome.

One of the basic conditions for the use of introgression lines as donors is their cytological stability, because it is closely connected with normal plant ontogenesis. Based on the fact that the lines analyzed were obtained by means of backcrosses from a large number of self-pollinating generations, it is very likely that they are meiotically stable. To acknowledge this, a cytological analysis of 20 resistant lines with reaction types 01, 1 and 2 was performed. For the establishment of cytological stability, chromosome pairing in metaphase I of meiosis in 3-4 plants from every line was studied. The number of the cells studied in each line varied from 100 to 140. The lines

Table 1

Characteristics of the population of *T. aestivum/T. miguschovae* introgression lines for resistance to leaf rust in 2016–2018

Cross combination	Number of lines with reaction type to leaf rust				
	01	1	2	3-4	1/3
MB	5	6	6	4	3
MK	15	11	12	6	2
MKS	10	6	5	12	3
Total	30	23	23	22	8

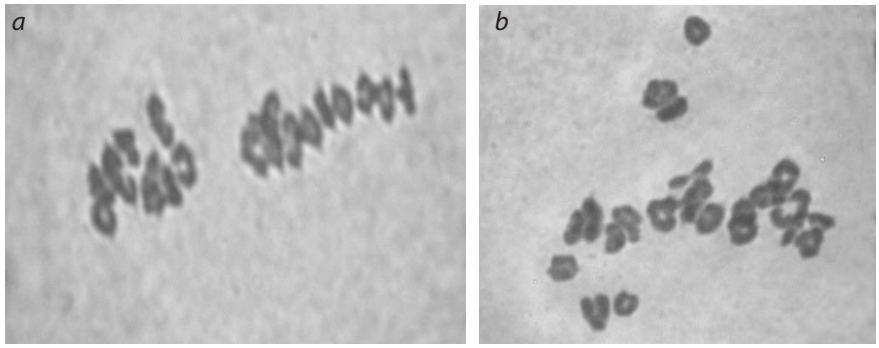

Figure 1. Association of chromosomes in metaphase I of meiosis in lines MK509-21^{II} (*a*) and MB1801-21^{II} (*b*).

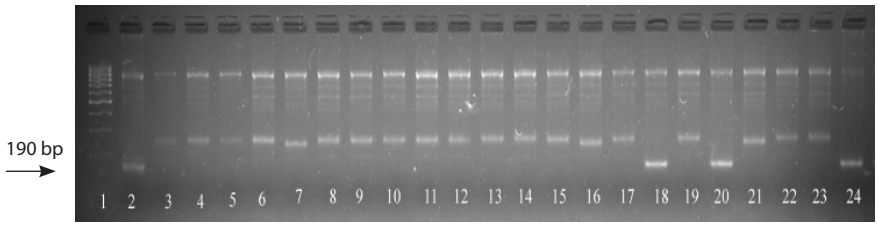

Figure 2. Products of amplification with the use of the GDM35-R and GDM35-L primer pairs to the diagnostic marker linked to *Lr39, a* gene for resistance to leaf rust. 1, marker of length; 2, *TcLr39*; 3, variety 'Avrora'; 4-23, lines; 24, *T. miguschovae*; 18, line MK452; 20, line MKC985.

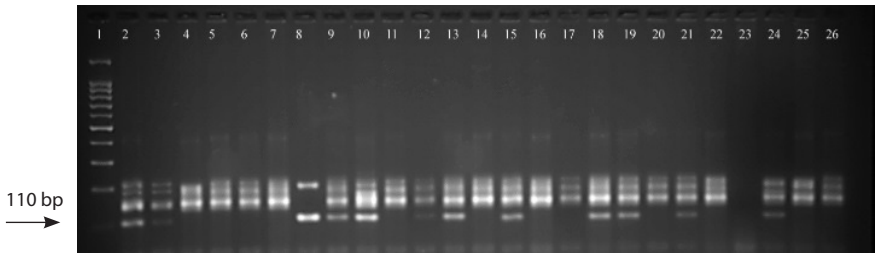

Figure 3. Products of amplification with the use of the *GDM87-L/R* primer pair to the diagnostic marker linked to the leaf rust resistance gene *Lr50.* 1, marker of length; 2, *T. miguschovae*; 3, KS96WGRC36 *(Lr50)*; 4, variety 'Bezostaya 1'; 5-26, the lines obtained on the basis of *T. miguschovae*.

were cytologically stable where they had more than 90 % of cells with the bivalent (21^{II}) configuration of chromosomes (Figure 1).

All lines analyzed formed the 21^{II} bivalent and were cytologically stable regardless of their origin and degree of resistance. The efficiency of selection for resistance to diseases depends on the presence of information about identification of genes. The genes the resistance to leaf rust *Lr18* and *Lr50* were transferred from *T. timopheevii* to common wheat, and *Lr21*, *Lr32*, *Lr39*, *Lr41*, *Lr42*, *Lr43* were transferred from *Ae. tauschii* (McIntosh, 2005). It is possible that *T. miguschovae* and the lines obtained on its basis carry these genes. Of these resistance genes, *Lr39* and *Lr41* are effective and *Lr50* is partially effective in various regions of Russia nowadays (Zhemchuzina, Kurkova, 2010). At the same time, the identity of genes *Lr39* and *Lr41* was established (Singh, 2004). In this connection, a screening of 76 resistance lines with genetic material from *T. miguschovae* for the presence of molecular markers linked to the *Lr39* and *Lr50* genes was performed. For identification of *Lr39*, a gene for resistance to leaf rust, the microsatellite marker *GDM35* was used. *GDM35* is widely used for screening varieties and lines in the USA (Sun et al., 2009). Specific fragments of amplification 190 bp to the diagnostic marker GDM35, linked to the *Lr39* gene were detected in the positive control line KS86WGRC02, the synthetic form *T. miguschovae*, and two highly resistant lines, MK452 and MKC985 (Figure 2).

Identification of the leaf rust resistance gene *Lr50* was done with two microsatellite markers, *Xgwm382* and *Xgdm87*, which outflank the *Lr50* locus at a distance of 6.7 and 9.4 cM, respectively. The 110-bp fragment was determined after amplification with the use of the *GDM87-L/R* primers, and the 139-bp fragment appeared after amplification with the use of the *WMS382-L/R* primers. The presence of the *Lr50* gene in the diagnosed sample was established upon revealing fragments of amplification of both markers.

The 110 bp fragment of amplification using the diagnostic marker *GDM87* linked to the resistance gene *Lr50* was identified in the positive control KS96WGRC36, the form *T. miguschovae* and in 32 resistance lines. The products of amplification of 22 lines are presented in Figure 3. The fragment specific to a marker linked to the *Lr50* gene was revealed in lines MK295, MK425, MK538, MKS627, MB1681, MK1795, MB1885, MB2305, MK2497, and MB2519 (Nos. 8, 9, 10, 12, 13, 15, 18, 19, 21, and 24, respectively).

4. Conclusions

The results obtained indicate the value of the studied lines for breeding for resistance to leaf rust. They are meiotically stable, different in resistance and can probably carry genes for resistance to leaf rust other than the effective genes *Lr39* and *Lr50*. Genetic material from *T. miguschovae* was identified in introgression lines with the use of C-banding and FISH methods.

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Conflict of interest. The authors declare no conflict of interest.